WS14.1 Ivacaftor potentiates mutant CFTR forms associated with residual CFTR function

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Objectives: Clinical studies have shown that ivacaftor, an oral CFTR potentiator, increased CFTR channel activity and improved lung function in patients with CF who have the *G551D-CFTR* gating mutation. The aim of this *in vitro* study was to evaluate whether ivacaftor potentiates mutant CFTR with non-gating CFTR channel defects.
Methods: The pharmacological action of ivacaftor on over 50 mutant CFTR forms was evaluated in electrophysiological studies using a panel of Fischer rat thyroid cells or human bronchial epithelia cells isolated from patients with CF.
Results: Ivacaftor potentiated multiple mutant CFTR forms that deliver sufficient amounts of functional CFTR to the cell surface to result in residual baseline chloride transport. These included *CFTR* mutations that result in mild defects in CFTR processing and delivery to the cell surface, reduced channel conductance, or reduced CFTR synthesis. In addition, ivacaftor also potentiated mutant CFTR forms that result in residual chloride transport due to defects in both channel gating activity and channel conductance, such as R117H-CFTR. Single-channel studies indicated that ivacaftor increased the channel open probability of R117H-CFTR to enhance chloride transport. In contrast, a minimal ivacaftor response was observed in cells expressing mutant CFTR forms associated with minimal baseline chloride transport.
Conclusions: This *in vitro* study indicated that ivacaftor acts on multiple mutant CFTR forms with defects beyond channel gating and supports investigation of the potential clinical benefit of ivacaftor in patients with CF who have cell surface CFTR that results in residual chloride transport.
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WS14.2 Efficient delivery of the human CFTR gene to pig lungs

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Pigs share many features of lung biology with humans, including anatomy, electrolyte transport, and immune responses. Models of neonatal CF pigs displayed a high degree of similarity in electrophysiological and pathological changes to human neonates with CF. The main objective of this study is to develop a pre-clinical model for delivery of the CFTR gene. In this study, we used wild-type pigs to investigate the technique of gene delivery using helper-dependent adenoviral (HD-Ad) vectors. HD-Ad vectors do not contain any viral coding sequences, and therefore inflict much weaker host immune responses. We have previously shown that this type of vector can be used to deliver CFTR gene to lungs of CF knockout mice and protect the mice from acute bacterial infection. Here we showed that aerosol delivery of the vector using an Aeroprobe catheter under flexible bronchoscopic guidance was well-tolerated by all subjects. A robust level of reporter expression was detected. Interestingly, many submucosal gland cells, the major CFTR-expressing cells in the airway, were transduced. There was no change in lung function observed based on the measurements performed before and 30 minutes after the vector delivery. To examine whether this method can be used for CFTR delivery, we aerosolized a HD-AD-CFTR vector into pig lungs at different dosages. We have observed dose-dependent and efficient transgenic CFTR expression in the pigs that received vector delivery. These results suggest that highly efficient delivery of HD-Ad vectors to the airway of large animals can be achieved and will provide important insights into the design of clinical studies for CF lung gene therapy.

WS14.3 Human amniotic mesenchymal stem cells can partially correct the cystic fibrosis phenotype upon coculture with F508del airway epithelial cells

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Stem cells may be beneficial for regenerating injured airway epithelium in cystic fibrosis (CF) lung disease. We evaluated human amniotic mesenchymal stem cells (hAMSCs) obtained from end term placentae for their ability to differentiate *in vitro* in airway epithelium and correct the CF phenotype. Real-time PCR showed that hAMSCs expressed at low levels CFTR mRNA and γ but not α and β subunits of ENaC. Cytofluorimetric analysis of cocultures of hAMSCs with F508del homozygous CFBE41o cells seeded at different ratios onto semi-permeable filters demonstrated that at least 50–80% of hAMSCs acquired a detectable CFTR expression on the apical membrane above the background. Confocal microscopy showed that CFTR was highly expressed on the apical membrane of hAMSCs. Fluorimetric measure of ion chloride efflux allowed to detect an increased function of the CFTR channel in cocultures as compared with CFBE41o– cells and hAMSCs alone. Amiloride-dependent fluid absorption decreased when CFBE41o– were cocultured with hAMSCs respect to CFBE41o– cells alone. Overall, these data show that hAMSCs are capable to resume some pathological features of CF airway epithelial cells, although the cellular and molecular mechanisms have to be deciphered.

WS14.4 Correction of both NBD1 energetics and domain interface is required to restore ΔF508 CFTR folding and function

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The folding and misfolding mechanism of multidomain proteins remains poorly understood. Although thermodynamic instability of the first nucleotide binding domain (NBD1) of ΔF508 CFTR partly accounts for the mutant channel degradation in the endoplasmic reticulum and is considered as a drug target in cystic fibrosis, the link between NBD1 and CFTR misfolding remains unclear. Here, we show that ΔF508 further compromises the marginally stable isolated NBD1 thermodynamically and kinetically. Although introduction of NBD1 stabilizing second site mutations was sufficient to increase the wild-type CFTR folding efficiency, restoring the ΔF508 NBD1 energetics failed to correct the ΔF508 CFTR biogenesis. Instead, second site mutations that stabilized both ΔF508-NBD1 energetic and the NBD1-MSD2 (membrane-spanning domain 2) interface interactions are required for wild-type like folding, processing, and transport function, suggesting a synergistic role of NBD1 energetics and topology in CFTR-coupled domain assembly. Identification of distinct structural deficiencies may explain the limited success of ΔF508 CFTR corrector molecules and suggests structure-based combination corrector therapies. These results may also serve as a framework for understanding the mechanism of interface mutation in multidomain membrane proteins.